



## The Effects of administration of different parts of banana (*Musa cavendish*) fruit extracts and peel powder on the oxidative/antioxidative characteristics and some mineral concentrations in neonatal dairy calves

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### ABSTRACT

The present study examined the effects of overripe banana (*Musa cavendish*) pulp and green banana peel extract and powder on oxidants/antioxidants parameters and some minerals in Holstein dairy calves. Forty newborn calves were randomly divided into four groups of 10 (control, group one, group two and group three). The groups were homogenous for the parity of the dams and the time of birth. Within 12-48 hours of birth, calves were assigned to their treatment groups. In the control group, animals received no banana meal. In group one, calves were supplemented with 2 g (dry matter)/kg body weight/day of overripe banana pulp extract for five days. The calves in group two were supplemented with 1 g (dry matter) of overripe banana pulp extract /kg body weight/day and 1 g (dry matter) of green banana peel extract/kg body weight/day for five days. The animals in group three were supplemented with 2 g/kg body weight/day of green banana peel powder for five days. Blood samples were taken on day 0 (at birth) and on days 7, 15 and 30 through the jugular vein. Age (the time of sampling) had a significant effect on the values of phosphorous, potassium, iron, copper, FRAP and activity of GPx enzyme ( $p < 0.05$ ). Significant group and sampling time interaction was seen for the FRAP concentrations ( $p < 0.05$ ). In conclusion, banana supplementation in the Holstein dairy calf's diet at the concentration and duration that was reported in the present study had beneficial effect on the values of FRAP.

### Keywords

Antioxidants, Banana, Calf, Minerals

### Abbreviations

MDA: malondialdehyde  
SOD: superoxide dismutase  
GPx: glutathione peroxidase

## Introduction

Calves are the substitution stocks for cows and bulls in a herd and their initial growth is the most important phase of their life. The nutrition of calves is an important factor in their health. For this reason, the diet of calves has been supplemented with many feed additives. Also, herbs are being used recently. The antioxidants of herbs may reduce the incidence of morbidity and mortality by reducing oxidative damage and improve the pre-weaning calf performance [1].

Banana is one of the most important tropical fruit crops, which belongs to the order of Zingiberales, the family of *Musaceae* and genus *Musa* [2]. Banana can be classified into commercial and non-commercial cultivars. The non-commercial ones are also referred to as indigenous varieties because their cultivation for export or trade is rare [3]. Non-commercial bananas which are cultivated in the south, the east and the southeast of Iran can be utilized as animal food. The use of natural products in the ration of food for animals results in the reduction of the presence of chemical residues in human foods [4]. All different parts of the banana plant including fruits, peels, leaves, roots, bulbs, flowers, gels and barks have medicinal utilizations [5, 6].

Bananas contain high levels of minerals such as potassium and phosphorus. The pulp and the peel possess various antioxidants including phenolic compounds such as catechin, epicatechin, lignin, tannins, anthocyanins, vitamins (A, B, C and E) and  $\beta$ -carotene. Forty percent of the total weight of fresh bananas

is peel which is considered as a rich source of protein, crude fat, lipid, dietary fiber, pectin, micronutrients, polyunsaturated fatty acids, and essential amino acids. Different studies have been conducted on the effects of various parts of banana plant supplementation in humans, small and large laboratory animals, chickens and even in prawns. In these studies, the effects of adding banana meal to ration have been evaluated on health, growth performance, feeding behavior, milk production, carcass characteristics, hematological, biochemical and immunological factors, diseases like metabolic disorders, and wound healing [4, 7-31]. *In vitro* studies have also been done for assessing the antimicrobial effects of bananas [5, 6, 32-36].

To the best of our knowledge, there is just one study on calves which has evaluated the effect of dietary supplementation of bananas on immunocyte populations [7]. Hence, this study was conducted to investigate the effects of dietary supplementation of extract of overripe banana pulp, extract of green banana peel with extract of overripe banana pulp together, and powder of green banana peel on oxidants/antioxidants variables and some mineral concentrations of Holstein dairy calves.

## Results

The treatment (group) had no significant effect on the measured variables ( $p < 0.05$ ). Age (time of sampling) had a significant effect on the values of phosphorous, potassium, iron, copper, FRAP, and activity of GPx enzyme ( $p < 0.05$ ). No significant effect was seen for calcium, sodium, zinc, MDA, and SOD. The

**Table 1**

The effects of treatments on measured minerals (LSM and SE) between trial groups.

LSM								
Parameter	Control	Group 1 <sup>1</sup>	Group 2 <sup>2</sup>	Group 3 <sup>3</sup>	SE <sup>4</sup>	Age	Group	Age $\times$ Group
Ca (mg/dL)	11.33	11.97	12.20	11.37	0.43	NS	NS	NS
P (mg/dL)	7.04	7.15	7.17	6.95	0.36	S	NS	NS
Cu ( $\mu$ g/dL)	71.22	69.44	67.59	67.52	4.80	S	NS	NS
Zn ( $\mu$ g/dl)	152.62	147.3	156.16	140.48	11.28	NS	NS	NS
Fe ( $\mu$ g/dl)	120.84	94.55	86.18	74.98	15.76	S	NS	NS
Na (mEq/L)	148.28	145.98	150.88	146.30	3.44	NS	NS	NS
K (mEq/L)	6.34	6.14	6.12	5.66	0.36	S	NS	NS

<sup>1</sup>: Overripe banana pulp extract supplemented group, <sup>2</sup>: Overripe banana pulp extract + green banana peel extract supplemented group, <sup>3</sup>: Green banana peel powder supplemented group, <sup>4</sup>: Standard error  
S: significant effect ( $p < 0.05$ ), NS: non significant effect, Ca: calcium, P: phosphorous, Cu: copper, Zn: zinc, Fe: iron, Na: Sodium, K: potassium

significant treatment and the sampling time interactions (treatment  $\times$  sampling time) were seen for the quantities of FRAP ( $p < 0.05$ ), while there were no

significant interactions for the other variables (Tables 1-3).

**Table 2**

The effects of treatments on oxidants/antioxidants characteristics (LSM and SE) between trial groups.

LSM								
Parameter	Control	Group 1 <sup>1</sup>	Group 2 <sup>2</sup>	Group 3 <sup>3</sup>	SE <sup>4</sup>	Age	Group	Age $\times$ Group
FRAP (mmol Fe2+/L)	2.35	2.29	2.36	2.36	0.06	S	NS	S
MDA (nmol/ml)	2.20	3.03	3.31	2.65	0.67	NS	NS	NS
SOD (Units/gr Hb)	13924	13619	13805	13729	233.89	NS	NS	NS
GPx (Units/gr Hb)	2390.66	2386.71	2445.11	2559.58	106.41	S	NS	NS

<sup>1</sup>: Overripe banana pulp extract supplemented group, <sup>2</sup>: Overripe banana pulp extract + green banana peel extract supplemented group, <sup>3</sup>: Green banana peel powder supplemented group, <sup>4</sup>: Standard error  
S: significant effect ( $p < 0.05$ ), NS: non significant effect, FRAP: Ferric Reducing Ability of Plasma, MDA: malondialdehyde, SOD: superoxide dismutase, GPx: glutathione peroxidase

**Table 3**

The effects of treatments on FRAP amount (LSM  $\pm$  SE) with a significant age and group interaction at various sampling time (age).

Age	Control	Group 1 <sup>1</sup>	Group 2 <sup>2</sup>	Group 3 <sup>3</sup>
FRAP (mmol Fe2+/L)				
Day 0	2.26 $\pm$ 0.08 <sup>a</sup>	2.14 $\pm$ 0.08 <sup>a</sup>	2.30 $\pm$ 0.09 <sup>a</sup>	2.13 $\pm$ 0.08 <sup>a</sup>
Day 7	2.43 $\pm$ 0.08 <sup>a</sup>	2.41 $\pm$ 0.08 <sup>a</sup>	2.55 $\pm$ 0.09 <sup>a</sup>	2.44 $\pm$ 0.08 <sup>a</sup>
Day 15	2.28 $\pm$ 0.08 <sup>a</sup>	2.33 $\pm$ 0.08 <sup>a</sup>	2.29 $\pm$ 0.09 <sup>a</sup>	2.61 $\pm$ .08 <sup>b</sup>
Day 30	2.42 $\pm$ 0.08 <sup>a</sup>	2.28 $\pm$ 0.08 <sup>a</sup>	2.30 $\pm$ 0.09 <sup>a</sup>	2.27 $\pm$ 0.08 <sup>a</sup>

<sup>1</sup>: Overripe banana pulp extract supplemented group, <sup>2</sup>: Overripe banana pulp extract + green banana peel extract supplemented group, <sup>3</sup>: Green banana peel powder supplemented group  
Means within rows lacking a common superscript, were significantly different ( $p < 0.05$  or  $0.05 < p < 0.1$ )

## Discussion

Levels of iron significantly increased from birth to day 30 which is consistent with the results of another study in calves [37], but in contrast with the report from Mohri et al. (2007) [38]. The discrepancy may be resulted from the influence of body iron reserve and the iron content of the diet. In the present study, the concentration of potassium declined on day 30 compared with its amounts at birth except in group 3 be-

cause of high potassium content (78.1mg/g) of banana peel [39, 40]. The decreased value of potassium, can be justified by a significantly lower tubular resorption of potassium in calves in postnatal period than sodium and chloride which are similar to the levels in adult animals [41].

In agreement with our results, Mohri et al. (2007) reported that phosphorus levels significantly increased by age [38]. This can be attributed to the enhancement of renal phosphate reabsorption by growth hormone, which is high in growing animals [42]. Copper value

significantly increased from birth to day 30, similarly in the study of Enjalbert et al. (2002) reporting that plasma copper concentration in calves increased after birth and reached its normal values at 3 weeks of age [43]. This can be attributed to the elevation in the concentration of liver copper in the first 2 months of life and the increased absorption of copper in newborn calves in comparison with adults in response to physiological needs for bone and connective tissue growth and development [44].

Age had a significant effect on the activity of GPx enzyme and the values of FRAP. The activity of GPx was significantly declined on day 30 compared with its activity at birth. Previous studies reported similar results and mentioned that age related changes in the antioxidants status can be detected in several species and several tissues, and factors such as nutrition and hormones have important effects on the activity of GPx [45, 46]. Micronutrients such as Zn, Cu, Fe and Mn improve the efficiency of antioxidant system [47], so the results obtained for the values of FRAP may be resulted from the significant increase in the amounts of Cu and Fe.

In the calves supplemented with unripe banana peel powder, the amounts of FRAP were higher than the other three groups on day 15. FRAP assay, is a novel method for evaluating the antioxidant power. The total FRAP is dependent on the individual plasma antioxidants (uric acid, ascorbic acid, vitamin E, bilirubin, albumin, and others) [48]. The reason for the higher value of FRAP in calves supplemented with peel powder compared with those supplemented with pulp extract is the higher antioxidant content of the peel. Although the amounts of ascorbic acid regardless of the ripening stage was constant (10 mg/100 g) in both peel and pulp [49]. The amounts of most other antioxidants are higher in the peel than pulp [50]. In the study of Fatemeh et al. (2012) the amounts of total phenolic and flavonoid contents were higher in the Cavendish variety, the green stage and the peel of banana than pulp [50]. Similarly, Sundaram et al. (2011) revealed that fruit maturation and ripening was accompanied by a decrease in the activities of phenolic compounds and antioxidant enzymes [51]. Devatkal et al. (2014) correlated the antioxidant capacity of bananas to their gallic catechin and dopamine contents [52]. They observed that gallic catechin was more abundant in the peel (158 mg/100 g dry wt.) than in the pulp (29.6 mg/100 g dry wt.). Gallic catechin is a polyphenol and a major catechin in the banana. Catechins are the strongest antioxidants in phytochemicals [50]. In the study of Kanazawa and Sakakibara (2000) on the antioxidants of *Musa cavendish*, large amounts of dopamine in both the pulp and the peel were found. The amounts of dopamine,

a strong water-soluble antioxidant in the peel (80-560 mg/100 g) were greater than the amounts in the pulp (2.5-10 mg/100g) and decreased a little with ripening. Flavanone glycoside naringin and flavonol glycoside rutin are two antioxidative phytochemicals in bananas that are mostly in the peel (10 mg) compared with the negligible amounts in the pulp [49]. Also, carotenes and tocopherols were appreciable in the peel and less in the pulp [50]. These reports are in agreement with our results in the group three in which the FRAP values were more than that of calves supplemented with pulp. Furthermore, in calves supplemented with unripe banana peel powder the FRAP values were higher in comparison with those in group two. It seems that the causative agent may be the differences in processing techniques for preparing unripe banana peel supplement (extract or powder) which result in the reduction of the antioxidants in the extract.

Treatment did not have any significant effects on the activity of MDA and the antioxidant enzymes of SOD and GPx. Giri et al. (2016) reported that in Labeo rohita (is a species of fish belonging to Cyprinidae family) supplemented with yellow banana peel flour for 60 days, the activity of antioxidant enzymes SOD and GPx were significantly higher and MDA activity was significantly lower compared with the control group [53]. In prawns supplemented with green banana peel extract for 120 days a significant increase in SOD activity has been observed [30].

Change in antioxidant parameters and lipid peroxidation happens more slowly in RBCs than in the plasma or serum [54]. On the other hand MDA that is generated from lipid peroxidation is known as a marker of oxidative stress in cells [55]. Thus, it can be concluded that after antioxidant supplementation (and increasing FRAP amount in serum), antioxidative defense in cells (GPx and SOD) and consequently the amount of MDA change slowly. In addition, it is important to note that in neonatal calf erythrocyte, the pro-oxidant features predominate over antioxidant features which make it susceptible to oxidative damage. As a result of higher autoxidation, the amounts of peroxide, hydroxyl and superoxide radicals produced from foetal haemoglobin in neonatal erythrocytes are more than these generated from adult haemoglobin. Furthermore, in newborn the activities of GPx and catalase enzymes and the ability to renew fatty acids are reduced and the amounts of vitamin E in young animals' erythrocytes is inadequate which has an important role in preventing membrane lipids autoxidation [56]. As a result of lower antioxidative defense in neonatal calves' RBCs, ROS and lipid peroxidation products including MDA are increased [55, 56]. Moreover, consumption of solid feed in growing calves results in a period of oxidative stress from 2nd

week of life [54].

In addition, in the present study the duration of supplementation with banana (pulp and/or peel, extract or powder) was short in comparison with the previous studies that reported a reduction of MDA activity as a result of banana peel [53] or pulp supplementation [57]. All these reports are in agreement with our results which show no significant differences in MDA level between treatment groups and control and also unchanged GPx and SOD activity.

In conclusion, banana supplementation in the Holstein dairy calf's diet at the concentration and duration that was reported in the present study had beneficial effects on the values of FRAP. Further studies are required to identify the optimal dosage and duration of banana supplementation in dairy calves.

## Material and methods

### Experimental design

The duration of study was seven months, from 18 August 2017 to 18 March 2018 in a dairy herd with about 210 calves per year at the suburbs of Mashhad (northeast of Iran). This herd consists of pure bred animals of Holstein breed. The herd was totally restricted in open-shed housing with no access to pasture. The ingredients of dry cow ration in both far off dry period and close up dry period are shown in Table 4. The ration was balanced according to NRC recommendations (NRC, 2001).

After parturition, the umbilicus of each calf was treated with povidone iodine and they were allowed to remain with their dams until the umbilicus dried off. The calves were weighed, then their sex was recorded and they were transferred to individual pens bedded with straw. Within the first 6 hours of life, the calves were fed dam's colostrum by nipple bottle in amounts of 10% of their body weight and the colostrum feeding was repeated every 8 hours for 48 hours. Then, herd milk was replaced for feeding twice daily (2 kg every 12 h) until seventy days of life. The calf starter ration was also balanced according to NRC recommendations (NRC, 2001). After transferring them to an individual pen, the animals had free access to clean drinking water. The calves were weaned at seventy days of life.

Forty newborn Holstein dairy calves from both sexes were selected for the study. The animals were randomly divided into four groups of ten. The groups were homogenous for parity of dams and time of birth. In the control group, animals received no banana meals. In group one, calves were supplemented with 2 g (dry matter)/kg body weight/day of overripe banana pulp extract for five days. Calves in group two were supplemented with 1 g (dry matter) of overripe banana pulp extract /kg body weight/day and 1 g (dry matter) of green banana peel extract/kg body weight/day for five days. In group three animals were supplemented with 2 g/kg body weight/day of green banana peel powder for five days. The amounts and duration of supplementation were selected according to a previous report in calves (7). The extract or powder was mixed with milk or warm water and was administered to the calves orally in a milk bottle. All other aspects of their diets were identical for all groups including the control group.

### Preparation of aqueous extracts

Ripe and also completely green Bananas (*Musa cavendish*) were purchased locally from a banana local market without any ethylene gas exposure and were stored at 20 °C for 24 h before

extraction.

### Preparation of green banana peel extract

Green bananas were rinsed thoroughly in tap water, surface sterilized with 70% alcohol and then they were rinsed by distilled water to remove any contaminants. Peels were manually separated from the pulp and they were put into 70 °C water for 20 second to inactivate polyphenol oxidases. The peels were cut into small pieces by using a sharp knife and they were dried in an oven at 60 °C for 38 h. Then, the dried peel was ground into a powder with an industrial grinder. The milled peel was mechanically stirred for 2 h (1 g in 10 ml distilled water) in a vacuum evaporator under reduced pressure at 60 °C. After extraction, the extract was centrifuged for 15 min at 3500 rpm. The supernatant containing the water-soluble extracts was transferred into 50 ml falcon tubes and it was stored at -70 °C until the experiment started.

### Preparation of overripe banana pulp extract

Yellow bananas were left at room temperature until peels became yellow brown and the edible portion became leaky (overripe). The peels were separated from the pulps by hand. The flesh was weighed, cut into appropriate sizes, and mixed with a 3-fold weight of deionized water in a vacuum evaporator under reduced pressure at 60 °C for 10 h. The homogenate was centrifuged at 1500 rpm for 15 minutes. The obtained supernatant was transferred into 50 ml falcon tubes and was stored at -70 °C until the experiment started.

### Preparation of green banana peel powder

The peels were manually separated from the pulp and were cut into small pieces. Pieces were shade-dried for about two weeks and then they were crushed to make a coarse powder in a pulverizer. The powder was stored in cold, dry and dark place until the experiment started.

### Calculating dry matter

The moisture content of the extracts was calculated on the basis of weight loss after the sample had been heated in an oven at 105 °C. The DM content of pulp and peel extracts were 45.86% and 8.5%, respectively.

### Blood sampling

The blood samples were taken on day 0 (at birth) and on days 7, 15, and 30 through the jugular vein with the aid of disposable syringes. 2.5 milliliters of blood was transferred into EDTA-3K tubes for hematological analysis and hemolysate preparation and 7.5 ml was transferred to plain tubes for serum separation. As soon as collection done, all tubes were placed on ice and were immediately transferred to the laboratory. The blood in the plain tubes was allowed to clot at room temperature and then it was centrifuged for 15 min at 3000 rpm for serum separation. The serum was aliquoted into 1.5 ml microtubes and the sample code was written on them. The serum was frozen at -20 °C until analysis.

### Minerals measurement

Calcium (Ca), phosphorous (P), iron (Fe), sodium (Na), potassium (K), copper (Cu), and zinc (Zn) concentrations in serum were measured by commercial colorimetric kits (for Ca, P and Fe: Pars Azmoon, Tehran, Iran and for Na, K, Cu and Zn: DIALAB, Wiener Neudorf, Austria) using an autoanalyzer (Mindray, BS-200E, Shenzhen, China). The control serum (Centronorm, centronic GmbH, Wartenberg, Germany) was used for controlling measurement accuracy. The inter assay and intra assay of methods were less than 5%.

Estimation of serum oxidant/antioxidant characteristics

MDA in the serum samples was estimated by reaction with thiobarbituric acid (TBA) at a high temperature to generate a MDA-TBA adduct. The pink color adduct was measured spectrophotometrically at 530-540 nm (Nalondi assay kit, Navand Salamat, Orumieh, Iran). Kit sensitivity was 0.1 nmol/well.

Measurement of Ferric Reducing Ability of Plasma (FRAP): Antioxidant potential in the serum samples was measured through reduction of ferric iron (Fe<sup>3+</sup>) to ferrous iron (Fe<sup>2+</sup>) by single electron-transfer mechanism by antioxidants present in the serum. Change of color of the reaction was estimated colorimetrically at 593 nm (Naxifer assay kit, Navand Salamat, Orumieh, Iran). The antioxidant potential of the samples was determined using a ferrous iron standard curve. Kit sensitivity was 2 µmol Fe<sup>2+</sup>.

Hemolysate preparation

Blood samples in the anticoagulant containing tubes were centrifugated at 3000 rpm for 5 minutes. The plasma and buffy coat layers were removed immediately and normal saline (0.9% NaCl) with approximately two times the volume of the packed RBC that was added for washing. Then, the tubes were centrifugated at 2000 rpm for 5 minutes. The washing process was repeated four times. After the 4th washing, cold redistilled water was

added to the washed RBC in a ratio of 3:1 and vortexed vigorously to make hemolysate. After final centrifugation, the hemolysate was refrigerated at 4 °C for 15 min and then it was aliquoted to 1.5 ml micro tubes on which the sample code was written and they were frozen at -20 °C until analysis.

Estimation of antioxidant enzymes activity

The activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured colorimetrically in hemolysate samples using Ransod and Ransel commercial kits, respectively (Randox Co., Crumlin, United Kingdom). The inter assay and intra assay of SOD measurement method were 7.07% and 3.58%, respectively and the sensitivity was < 6.13 U/ml. The total assay and intra assay of GPx measurement method were 4.37% and 3.2%, respectively, and the sensitivity was 75 U/L.

Statistical Analysis

The data was subjected to repeated measure analysis of variance (ANOVA) using the PROC MIXED of SAS 9.2 (SAS institute Inc.). Normality of all variables was evaluated by univariate procedure. The outcome variables with Shapiro-Wilk values of ≥ 0.05 were considered as normal (FRAP, phosphorous, sodium) and all the other variables were transformed by using a natural logarithmic transformation to reach a normal distribution. The time of sampling (0, 7, 15 and 30), group (control, group one, two and three), sex and parity of dams were used as independent effects

Table 4  
The ingredients of dry cow ration in both far off dry period and close up dry period.

Ingredients	Far off dry period ration (%)	Close up dry period ration (%)
Alfalfa hay	17.79	13.71
Corn silage	25.22	19.45
Wheat straw	31.16	13.73
Barley grain rolled	10.37	16
Corn grain ground dry	5.73	16.68
Wheat bran	7.7	6.14
Fish meal	1.27	-
CaCo3	0.23	-
Salt	0.24	-
Canola meal	-	2.67
Soy meal, expellers	-	6.59
Anionic supplement	-	3.92
TMS	-	1.11
Vitamin/mineral supplement*	0.29	-
Total	100	100
Calculated dry matter intake	14 kg	17 kg

\*Supplements contain/kg: Vit A 1,000,000 IU, Vit D3 300,000 IU, Vit E 10,000 IU, Ca 6118 mg, P 1500 mg, Mg 5000 mg, Mn 1000 mg, Zn 1000 mg, Cu 500 mg, Se 50 mg, Iodine 50 mg, Fe 1000 mg, Co 5 mg and antioxidant 1000 mg.

and all measured variables were considered as dependent. The results were expressed as Least squares means (LSM) ± standard errors of means (SE) in each group. The effects of independent factors were considered significant at *p* < 0.05, whereas a trend toward significance was noted at 0.05 < *p* < 0.1.

Animal Welfare Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received (3/41677). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

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Author Contributions

N.K.R contributed to the main design of study, sample collection, laboratory tests, data analysis and drafting the manuscript. M.M contributed to main design of the study, data analysis and reviewed and edited the manuscript. H.A.S and A.H. contributed to the main design of the study. All authors approved the final version of the manuscript for publication.

Conflict of Interest

The authors declare they have no conflict of interest.

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